wherein the [chromatin-associated protein comprises] factors ~~provided by the~~ eukaryotic cells comprise ~~HMG1~~ chromatin-associated protein.

60. (Amended) A method [according to claim 58,] of promoting beta recombinase activity comprising the step of providing beta recombinase with eukaryotic cell factors which the beta recombinase is capable of using in order to exhibit recombinase activity;

wherein the [~~factors~~ provided by the] eukaryotic [cells] cell factors comprise HMG1 chromatin-associated protein.

REMARKS

The Official Action dated August 16, 2000, has been carefully considered. Accordingly, the changes presented herewith, taken with the following remarks, are believed sufficient to place the present application in condition for allowance. Reconsideration is respectfully requested.

By the present amendment, nonelected Claims 29, 30 and 51 have been cancelled and Claims 27, 28, 33, 38-40, 52, 53, 55 and 60 have been amended. Support for the amendments to Claims 27 and 28 may be found at page 2, lines 24-29. Support for the amendment to Claim 33 can be found at page 4, lines 14-15 and page 13, lines 10-11. Claims 38-40 have been amended to change their dependency and Claims 39 and 40 have also been amended to define the target sequences in accordance with the specification at page 1, lines 11-12, page 2, lines 28-29, and page 3, lines 29-31. Support for the amendment to claim 52 may be found at page 5, line 23-page 6, line 7, and page 6, lines 9-28. Finally, Claims 53, 55 and 60 have been amended to stand in independent form. It is believed that the claims are fully supported by the specification

as originally filed and do not raise any new issue requiring further consideration or search. Accordingly, entry is believed to be in order and is respectfully requested.

In the Official Action, Claims 27, 28, 31-50, 52, 54 and 56-59 were rejected under 35 U.S.C. §112, first paragraph, as not supported by an enabling specification. The Examiner asserted that the specification, while being enabling for the method of using beta recombinase in mammalian cells containing HMG1 protein, does not reasonably supply enablement for all eukaryotic cells. The Examiner alleged there is a strict requirement for Hbsu protein, E. coli. HU protein and/or mammalian HMG1 protein for the site-specific recombination catalyzed by beta recombinase. The Examiner further alleged that neither the prior art nor the Applicants demonstrate that the beta recombinase is capable of mediating site-specific recombination in the absence of one of these proteins, therefore, any eukaryotic cell lacking expression of one of these proteins would be incapable of a recombinase event.

However, as will be set forth in detail below, Applicants submit that the methods defined by claims 27, 28, 31-50, 52, 54 and 56-59 are fully enabled to one of ordinary skill in the art in accordance with the requirements of 35 U.S.C. §112, first paragraph. Accordingly, this rejection is traversed and reconsideration is respectfully requested.

More particularly, according to Claim 27, the invention is directed to methods for mediating intramolecular recombination selected from deletions of DNA fragments located between two *six* sites and inversions of DNA fragments located between two *six* sites in eukaryotic cells. The methods comprise the step of providing eukaryotic cells with prokaryotic beta recombinase and its specific target sequences. The prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to exhibit recombinase activity.

According to Claim 28, the invention is directed to methods for mediating intramolecular recombination selected from deletions of DNA fragments located between two *six* sites and inversions of DNA fragments located between two *six* sites in chromatin structures of eukaryotic cells. The methods comprise the step of providing eukaryotic cells with prokaryotic beta recombinase and its specific target sequences. The prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to exhibit recombinase activity.

According to Claim 43, the invention is directed to methods for catalysing site-specific resolution of DNA sequences in an extrachromosomal target introduced into a eukaryotic cell. The methods comprise the step of catalysing the site-specific resolution with beta recombinase. The eukaryotic cell provides factors which beta recombinase is capable of using in order to exhibit recombinase activity. According to Claim 58, the invention is directed to methods of promoting beta recombinase activity. The methods comprise the step of providing beta recombinase with eukaryotic cell factors which the beta recombinase is capable of using in order to exhibit recombinase activity.

Independent claims 27 and 28 recite limitations that the prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to exhibit recombinase activity, while independent claims 43 and 58 recite limitations that the eukaryotic cell provides factors which beta recombinase is capable of using in order to exhibit recombinase activity. Further, the specification, at page 8, line 30-page 9, line 1, teaches the use of HMG1/HMG2 chromatin-associated protein, B. Subtilis Hbsu protein and E. coli HU protein. The specification also teaches, at page 9, lines 3-30, how to determine whether eukaryotic cells provide the appropriate factors required for beta recombinase activity. One of ordinary skill would appreciate that suitable eukaryotic factors include HMG1/HMG2 proteins, Hbsu proteins and HU proteins. The

Examiner appears to be of the opinion that Applicants are claiming methods employing any eukaryotic cell. However, Applicants submit that the present methods employ a certain type of eukaryotic cell, namely those providing factors which the prokaryotic beta recombinase is capable of using to exhibit recombinase activity as recited in claims 27, 28, 43 and 58.

A disclosure is enabling if, from the information set forth in the specification, coupled with information known in the art, one of ordinary skill in the art could make and use the invention without undue experimentation, *United States v. Teletronics, Inc.*, 8 USPQ2d 1217, 1224 (Fed. Cir. 1988). Moreover, every aspect of a generic claim certainly need not have been carried out by an inventor, or exemplified in the specification; rather, reasonable detail must be provided in order to enable members of the public to understand and carry out the invention, *Genetech v. Novo Nordisk, A/S*, 42 U.S.P.Q.2d 1001, 1005 (Fed. Cir. 1997). As the specification teaches how to determine whether eukaryotic cells provide the appropriate factors required for beta recombinase activity and teaches the use of specific proteins, the specification enables the methods of claims 27, 28, 43 and 58. Accordingly, independent claims 27, 28, 43 and 58, and claims dependent thereon, are enabled in accordance with the requirements of 35 U.S.C. §112, first paragraph. It is therefore submitted that the rejection under 35 U.S.C. §112, first paragraph, has been overcome. Reconsideration is respectfully requested.

Claims 27, 28, 33, 35-42, 48, 50 and 52 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. However, as will be set forth below, Applicants submit that Claims 27, 28, 33, 35-42, 48, 50 and 52 are definite. Accordingly, this rejection is traversed and reconsideration is respectfully requested.

More specifically, the Examiner asserted that claims 27 and 28 are unclear in the recitation of "mediating intramolecular recombination" because while the specification supports

the use of beta recombinase and proper co-factors to mediate recombinase between two *six* sites, it is unclear how the addition of beta recombinase would mediate any and all recombination in eukaryotic cells. The Examiner also asserted that steps which specifically define the recombination events to be mediated by the addition of beta recombinase and the appropriate co-factors are needed.

Claims 27 and 28 now recite that the intramolecular recombination is selected from deletions of DNA fragments located between two *six* sites and inversions of DNA fragments located between two *six* sites. Moreover, the Examiner's attention is directed to page 2, lines 26-29 of the specification which teaches that beta recombinase catalyzes exclusively intramolecular deletions in inversions of DNA sequences located between two target sites for the recombinase, termed *six* sites, whereby one of ordinary skill would appreciate the meaning of "intramolecular recombination" and would be aware that appropriate factors include HMG1/HMG2, Hbsu and HU proteins. Thus, claims 27 and 28, when read in light of the specification, are definite.

The Examiner asserted that claim 33 is vague and unclear in its recitation of "two or more different specific recombination events at a time are promoted" as the specification teaches beta recombinase and the appropriate factors result in a recombination event between two intramolecular located *six* sites, but it is unclear how two or more different events could occur in this context, if the recombination is to occur between these two target sites, or if the recombination occurs at each site independently of another. Claim 33 now recites "whereby two related genes are inactivated" to clarify the method defined therein in accordance with the specification at page 4, lines 14-15 and page 13, lines 10-11.

The Examiner asserted that claims 39-40 are unclear in their recitation of "target sequences" as the specification teaches recombination only between *six* sites and no other

recognition sequences have been described, and without functional language that defines the direct repeat sequences as recognition sites for beta recombinase, it is unclear what is encompassed by "target sequences". Claims 39 and 40 now recite "DNA sequences containing *six* sites".

The Examiner asserted that claim 52 is unclear and incomplete and that steps which describe selection of mammalian cells containing the transgene are needed. Claim 52 now recites the additional steps of transfecting the cells with prokaryotic beta recombinase, and detecting the prokaryotic beta recombinase in cells, in accordance with the specification at page 5, line 23-page 6, line 7, which describes transfection conditions for forming transgenic cells, and page 6, lines 9-28, which discloses methods of detecting beta recombinase by immunofluorescence or immunoblotting.

It is therefore submitted that Claims 27, 28, 33, 35-42, 48, 50 and 52 are definite, whereby the rejection under 35 U.S.C. §112, second paragraph, has been overcome. Reconsideration is respectfully requested.

Finally, the Examiner objected to claim 57 under 37 C.F.R. 1.75 as being a substantial duplicate of claim 56. However, Applicants direct the Examiner's attention to claim 56, which recites that the beta recombinase promotes a deletion of DNA sequences located between direct repeated *six* sites, and claim 57 which indicates the beta recombinase promotes an inversion of DNA sequences located between inverted repeated *six* sites. As deletions and inversions are not the same, claims 56 and 57 are not duplicative. It is therefore believed that the objection should be withdrawn.

The Examiner indicated that claims 55 and 60 would be allowable if rewritten as independent claims. Accordingly, Claims 55 and 60 have been amended to stand in independent

form and are believed to be prima facie allowable. Moreover, as Claim 53 defines the factors provided by eukaryotic cells comprise HMG1 chromatin-associated protein, Claim 53 has also been amended to stand in independent form. It is therefore submitted that Claim 53 is also allowable.

Applicants appreciate the Examiner's acknowledgment that all claims are free from art.

It is believed that the above represents a complete response to the Examiner's rejections under 35 U.S.C. § 112, first and second paragraphs, and places the present application in condition for allowance. Reconsideration and an early allowance are requested. In the event that the present application is not in condition for allowance, entry of this amendment for purposes of appeal is requested.

Respectfully submitted,

Holly D. Kozlowski
Registration No. 30,468
Dinsmore & Shohl LLP
1900 Chemed Center
255 East Fifth Street
Cincinnati, Ohio 45202
(513) 977-8568

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